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Efficacy of Cidofovir in a Murine Model of Disseminated Progressive Vaccinia

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An animal model that mimics progressive disseminated vaccinia was elaborated. To this end nude (athymic) mice were inoculated intracutaneously with vaccinia virus in the lumbosacral area. Viral replication (DNA) in the skin was detected as early as day 2 postinfection (p.i.). Mice developed typical vaccinia lesions at the site of inoculation by day 4 to 6 p.i. By about 2 weeks p.i., the infection had spread all over the body, a situation reminiscent of disseminated vaccinia in humans. The infection resulted in viremia and spread of the virus to visceral organs, as well as to the brain. Topical treatment with cidofovir, initiated at the day of infection or at day 1 p.i., completely protected against virus-induced cutaneous lesions and against associated mortality. When treatment was initiated at a later time (day 2 to 5 p.i.), a partial but marked protective effect was noted, which can be explained by the fact that by that time, the virus had spread from the skin to the visceral organs. Next, infected animals were left untreated until the time (~2 weeks p.i.) at which disseminated vaccinia had developed. When systemic treatment with cidofovir was initiated at that time, it caused lesions to heal and regress. In most of these animals, lesions had completely (or almost completely) disappeared by day 10 to 15 after the start of therapy. The observation that cidofovir is able to cause healing of disseminated vaccinia lesions in animals should have implications for the therapy of complications of vaccination against smallpox.

The last case of smallpox occurred in 1977 in Somalia (the last laboratory infection occurred in 1978), and the World Health Organization declared the global eradication of smallpox in 1980. The subsequent discontinuation of vaccination against smallpox has rendered most humans vulnerable to smallpox infection. Virtually all children and many adults are now fully susceptible to smallpox. Following eradication of smallpox, every country that held variola virus, the causative agent of smallpox, should have destroyed the virus or transferred the stock(s) to one of two central repositories, i.e., the Centers for Disease Control and Prevention in Atlanta, Ga., or the State Research Center of Virology and Biotechnology (VECTOR laboratories) in Koltsovo, Russia. Because variola virus is very stable, even at −20°C, it may well be that certain countries have kept secret stocks of the virus. Illegally preserved stocks of smallpox virus could be used for biological or terrorist purposes. Such use could, in a highly mobile and susceptible population, cause a major epidemic (6).

Many countries are stockpiling the smallpox vaccine, the active component of which is the vaccinia virus (17). The United States recently started the vaccination of civilian health care and public health workers and military personnel (7). Vaccination against smallpox may result in a variety of complications, primarily due to the escape of the virus from the initial inoculation site (16). Adverse effects occur most often in first-time vaccinees (15). The number of vaccinated health care workers or military personnel presenting with adverse effects has been limited in this selected population (7). A large-scale vaccination campaign in the event of a bioterrorist attack may, however, result in a large number of patients presenting with adverse effects (3, 8, 23). The Global Advisory Committee on Vaccine Safety, a scientific advisory body of the World Health Organization, recently (June 2003) urged caution in the use of the vaccine, on the basis of two expert reports on the safety of smallpox vaccines. The committee concluded that "there is a real risk of serious adverse events following immunization with the smallpox vaccine" (www.who.int/vaccine_safety/topics/smallpox/en/). In mass vaccinations, the impact of vaccine reactions may constitute a significant health burden (8).

Most of the complications of vaccination against smallpox are the result of excessive replication of the virus (4). Consequently, potent and selective antiviral therapy may be valuable for the treatment of such conditions. Complications that result from excessive replication of vaccinia virus include accidental infection, generalized vaccinia, eczema vaccinatum, and progressive vaccinia (4, 18, 19). Images of complications of smallpox vaccination are available at www.bt.cdc.gov/agent/smallpox/vaccineimages.asp. In the case of accidental infection, vaccinia virus is transferred from the site of inoculation to other areas on the body of the vaccinee or a close contact (2, 3). If the virus disseminates through the bloodstream, it may result in generalized vaccinia. Persons with eczema (or other forms of atopic dermatitis), who have defects in the innate immunity of the skin, may be particularly prone to the spread of the virus (14). In individuals with defective cellular immunity, vaccination against smallpox may lead to progressive vaccinia, with an enormous enlargement of the primary vaccination lesion and eventually the development of vaccinia lesions at other places on the body (2).

To the best of our knowledge, there is at present no animal model that mimics such forms of complications of vaccination

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against smallpox and that would allow assessment of the efficacy of specific antiviral therapy. We therefore elaborated such a model. Intracutaneous infection of "nude" (athymic, lacking functional T cells) mice results in a generalized disseminated vaccinia, ultimately leading to the death of the animals. Although cidofovir is available from the Centers for Disease Control and Prevention under Investigational New Drug (IND) protocols for the treatment of severe adverse effects following smallpox vaccination (33), the activity of the compound in disseminated progressive vaccinia has not been demonstrated. We show here that cidofovir, which is licensed for the intravenous treatment of cytomegalovirus retinitis in AIDS patients, is able to cause healing of progressive disseminated vaccinia lesions.

MATERIALS AND METHODS

Compounds. Cidofovir was kindly provided by Gilead Sciences (Foster City, Calif.).

Virus. Vaccinia virus strain Lister (which was routinely used in Belgium as a vaccine against smallpox) was obtained from the Belgian "Rijksentstofinrichting" and cultured on human embryonic lung (HEL) cells.

Intracutaneous infection and treatment. Female athymic nude mice (Nude NMRI-nu or *nu*/*nu* mice, obtained from Elevage Janvier, Le Genest Saint Isle, France) were used throughout the experiments and were inoculated intracutaneously with vaccinia virus. Athymic nude mice, in which the development and differentiation of hair is severely impaired, lack functional T-cell immunity. Animals were placed in group housing and, to avoid interaction (of whatever kind) with the antiviral therapy, did not receive antibiotics. Animals received light ether anesthesia and were immobilized manually by one person. Scarifications were made by a second person. A 50-μl droplet of the viral inoculum (containing 5×10^5 PFU) was placed at the lumbosacral area. A sterile stainless steel blood lancet (Maersk Medical, Sheffield, United Kingdom) was used to produce a light scarification of $\approx$0.5 cm^2. The scarification was made in such a way that the skin did not bleed. Mice were either treated (i) systemically by subcutaneous injection of the appropriate dose of cidofovir (which was dissolved in 200 μl of prewarmed phosphate-buffered saline [PBS]) or (ii) topically at the lumbosacral area with 50 μl of cidofovir (1% in dimethyl sulfoxide [DMSO]). Mice were monitored daily for the development of vaccinia lesions and mortality. The experiments were approved by the Ethical Committee on animal vertebrate experiments at the University of Leuven.

Q-PCR. Blood was collected from anesthesized mice by heart puncture, and serum was isolated. Organs were dissected following extensive perfusion of the mice with PBS. Tissue samples were passed twice through cell strainers (Becton Dickinson Labware, Franklin Lakes, N.J.) and were homogenized in PBS (1:1 [wt/vol]). DNA was extracted from 25 μl of material by using the tissue protocol of the QIAamp DNA minikit (Qiagen, Hilden, Germany). Twenty microliters of RNase A (20 mg/ml) was added prior to the addition of buffer AL, and samples were incubated for 15 min to produce RNA-free DNA, after which the total DNA concentration was determined. Real-time quantitative PCR (Q-PCR) was performed on 4.5 μl of total DNA in a 25-μl reaction volume by using the TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, N.J.), forward primer 5′-AGA TCA TCG TAT GGA GAG TCG TAA GAT-3′ (final concentration, 300 nM), reverse primer 5′-TGA CTA CGT TGT TAT GAG TGC TTG GTA-3′ (final concentration, 300 nM), and a Taqman probe (6-FAM-ATC AAA ATA CAA GAC GTC GCT TTT AGC AGC TAA AAG AA-TAMRA; final concentration, 200 nM). The reaction was analyzed by using the real-time quantitative PCR apparatus SDS 7000 (Applied Biosystems, Foster City, Calif.). Plasmid DNA containing the amplified insert was used to prepare the standard curve and to quantitate the amount of viral DNA. The amount of viral DNA was normalized to the total amount of DNA in each sample. The detection limit was 10^{-6} ng of plasmid containing the vaccinia virus amplicon.

Detection of viral DNA in skin and serum. From day 1 to day 5, two intracutaneously infected *nu*/*nu* mice were sacrificed daily. A skin section of 1 cm^2 (at the lumbosacral area) was dissected and chopped. Twenty milligrams of tissue was used to extract total DNA. Total DNA was also extracted from 140 μl of serum. DNA from skin and serum was then analyzed by means of real-time Q-PCR for the presence of viral DNA.

Histology. Mice were killed by ether anesthesia and were transcardially perfused with PBS. Organs were fixed and embedded in paraffin, and sections were stained with hematoxylin and eosin according to standard procedures.

Statistics. The statistical significances of the mean day of death (MDD), the mean day of lesion appearance (MDLA), and the mean day of lesion healing (MDLH) were assessed by using a two-tailed Student *t* test. The statistical significances of the number of animals with lesions and the number of survivors were assessed by the Fisher exact test.

RESULTS

Disseminated vaccinia in athymic nude mice. Athymic nude (*nu*/*nu*) mice that had been inoculated intracutaneously with vaccinia virus at the lumbosacral area developed typical vaccinia lesions at the site of inoculation within the first several days after infection. Initial eruptions at the site of inoculation consisted of small vesicles that evolved to larger vaccinia lesions. Hairless immunocompetent mice inoculated in the same way also developed lesions, but those regressed spontaneously ~6 days after appearance (~10 days postinfection [p.i.]). In *nu*/*nu* mice, the enlarging vaccinia lesions often formed ulcers with necrotic tissue in the center and a raised, advancing rim. Lesions often merged and became confluent. Large crusts developed at a later stage. Skin and serum were analyzed every 5 days after infection. Viral DNA in the skin was detected as early as day 2 after infection, and levels increased steadily thereafter (Fig. 1). No viral DNA was detectable in the serum during the first 5 days (data not shown). About 2 weeks after infection, the lesions had spread; in most of the athymic nude mice, they had spread all over the body, a pattern reminiscent of disseminated cutaneous vaccinia in humans. Lesions were detected, for example, on the abdomen, head (ear, mouth, nose, etc.), and legs. Histologically, skin biopsies at day 20 p.i. revealed superficial ulcera with the disrupted epidermis replaced by a fibropurulent exudate (Fig. 2). The skin lesion was basically an ulcus with karyorhexis of epithelial cells and even some necrosis in the center of the ulcus. A transdermal inflammatory infiltrate of both polynuclear and mononuclear cells was present. At the edge, epithelial cells sometimes contained intranuclear viral inclusions. The neutrophilic infiltrate was likely a reaction to the necrosis. No evidence of bacterial infection of the ulcus was noted (Fig. 2). Animals usually became sick by week 4 to 5 after infection and succumbed (or were euthanized when moribund) during week 5 to 7 p.i. Organs of infected animals were dissected at day 30 p.i., and the presence of virus was assessed by real-time Q-PCR. Significant amounts of viral DNA were detected in the serum as well as the spleen, kidneys, liver, and lungs (Table 1). Although the virus was also detected in the brain (Table 1), at high levels in one animal, the mice did not present with obvious neurological symptoms. Histologically, despite the presence of viral DNA, no particular anomalies (such as viral inclusions) were observed in these organs, except for some microaggregates of neutrophils in the livers of two animals.

Effects of topical treatment with cidofovir. Cidofovir (a 1% solution in DMSO) was applied topically on the infected lumbosacral area. Treatment was initiated either on the day of infection (i.e., 2 h p.i.) or on day 1, 2, 3, or 4 p.i. and was continued for another 4 days (Table 2). Mock-treated animals were treated with DMSO only. The lightly scarified area healed rapidly in both mock-treated and cidofovir-treated an-

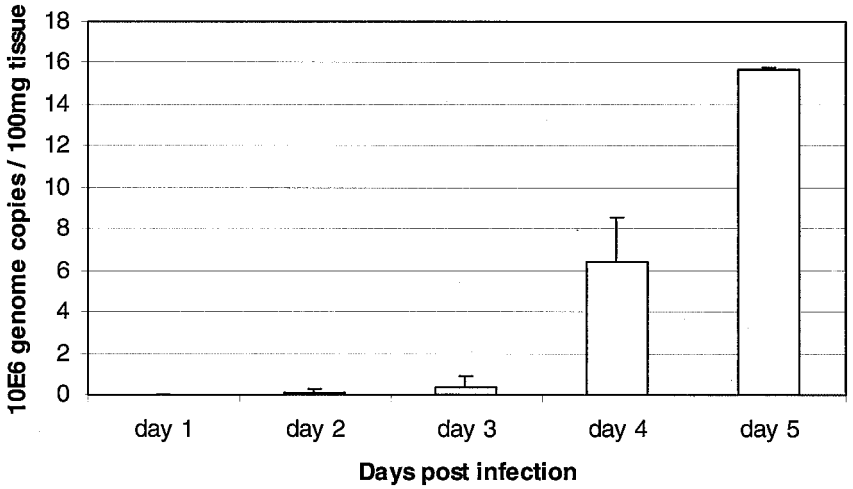

FIG. 1. Viral DNA levels in the skin of intracutaneously infected athymic nude (*nu/nu*) mice, as determined by Q-PCR in skin tissue at various days after infection (2 mice/condition).

imals. Mock-treated animals developed small lesions as of day 5 p.i. and larger lesions during the following days. Lesions progressed further and disseminated, and the animals died at day 34 ± 7 p.i. Treatment that was initiated either on the day of infection or on day 1 p.i. resulted in complete protection against disease progression, i.e., no vesicles or lesions developed for >127 days. When topical treatment was first initiated at day 2 p.i., only one animal developed lesions (initially on the mouth) as late as 49 days p.i. and died at day 94 p.i. When treatment was initiated on day 3 or 4 p.i. all animals ultimately developed lesions and succumbed. However, in these groups, the appearance of lesions and mortality were delayed relative to those for untreated animals (Table 2). Even when topical treatment with cidofovir was begun at 5 days p.i. (data not shown for an independent experiment), a delay of about 10 days in virus-induced mortality was noted (MDD, 32.8 ± 3.0 versus 42.2 ± 6.4 for mock-treated versus cidofovir-treated animals, respectively [$P < 0.05$; $n = 5$ animals per group]).

Effects of systemic treatment with cidofovir on disseminated vaccinia. We next assessed whether systemically (subcutaneously) administered cidofovir could prevent the appearance of vaccinia lesions when treatment was initiated 2 h after infection. Infected animals were treated with cidofovir at 100, 50, or 25 mg/kg of body weight/day for 2 periods of 5 consecutive days (interrupted by a 2-day drug-free period). The MDLA was 4.5 ± 0.8 days for the mock-treated group ($n = 6$). Mice in the 50- and 100-mg/kg dose groups were free of lesions during treatment, but lesions recurred some time after treatment was stopped. The MDLA for drug-treated animals was 36 ± 5.8 days ($P < 0.001$) for the 100-mg/kg/day condition ($n = 5$), 25 ± 16 days ($P < 0.05$) for the 50-mg/kg/day condition ($n = 5$), and 6.6 ± 1.5 days ($P < 0.05$) for the 25-mg/kg/day condition ($n = 5$) (data not shown).

We then studied whether cidofovir was able to confer protective activity if systemic (subcutaneous) treatment was first initiated at a time when the infection had already disseminated to other parts of the body. Lesions had disseminated to an important extent by day 15 p.i. (Table 3, experiment 1, and Fig. 3A); when left untreated, the animals died at 35 ± 0.6 days p.i. When treatment with cidofovir (given subcutaneously at 100 mg/kg/day for 21 days during a 24-day period) was started at day 15 p.i., a marked improvement in the severity of the lesions was observed as early as 3 to 5 days after the start of treatment and lesions had virtually completely healed by 13 to 14 days after the start of therapy (about 1 month after infection) (Table 3 and Fig. 3B). Following cessation of therapy, the disease recurred and animals succumbed (MDD, 51 ± 6 days [$P < 0.05$]). The healing effect of cidofovir on disseminated vaccinia was confirmed in another experiment (Table 3, experiment 2). Interestingly, marked protective activity was also observed with less-frequent dosing. Even one, two, or three weekly doses (initiated on day 15 p.i.) of cidofovir for 7 consecutive weeks resulted in marked improvement (although not necessarily

TABLE 1. Vaccinia virus DNA in various organs of *nu/nu* mice 30 days p.i.

Organ	No. of vaccinia virus genome copies/ml			
	Mouse 1	Mouse 2	Mouse 3	Mean ± SD
Brain	4.71E+05	1.27E+04	1.93E+04	1.68E+05 ± 2.62E+05
Liver	1.84E+04	2.77E+04	1.64E+04	2.09E+04 ± 6.05E+03
Kidney	3.59E+03	1.17E+04	3.10E+03	6.13E+03 ± 4.83E+03
Lung	3.80E+03	2.30E+04	5.78E+03	1.09E+04 ± 1.06E+04
Spleen	3.01E+03	3.27E+03	1.01E+03	2.43E+03 ± 1.24E+03
Serum	1.50E+05	1.98E+05	2.31E+04	1.24E+05 ± 9.03E+04

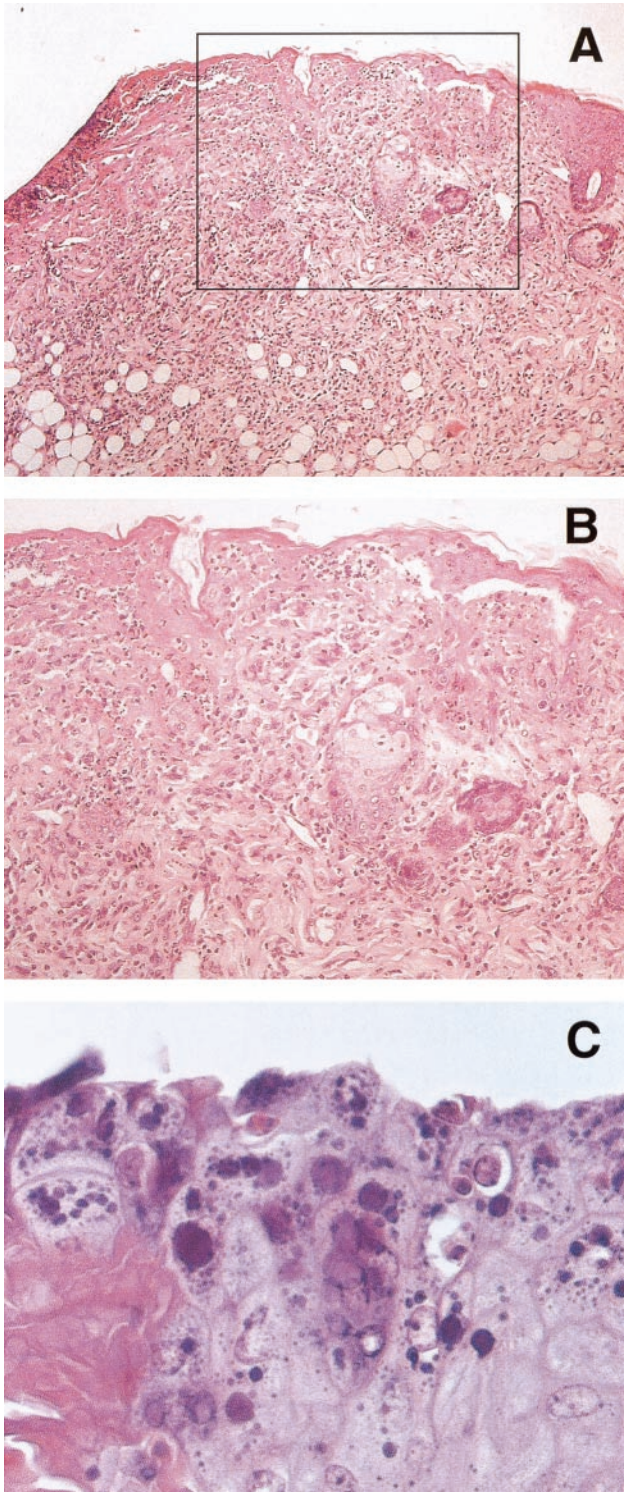

FIG. 2. Histological appearance of vaccinia skin lesions in athymic nude mice. (A) Section through lesion. The epidermis is disrupted, and a mixed inflammatory infiltrate is present throughout the dermis. (B) Further magnification indicates that the ulcer is covered by a fibropurulent exudate. (C) Typical viral inclusions are observed in several epithelial cells.

complete healing) of the vaccinia lesions as well as in a significant delay in virus-induced mortality.

DISCUSSION

Complications of vaccination with the smallpox vaccine are mostly related to excessive viral replication (2, 3, 6). Accidental infection with vaccinia can occur in immunocompetent patients, but complications of vaccination are most often seen either in immunodeficient patients or in patients with eczema or other forms of atopic dermatitis (14). People with defective cell-mediated immunity are indeed unable to control vaccinia replication once it has initiated. Patients with atopic dermatitis are unusually susceptible to the initiation and spread of vaccinia virus infection because of defective innate immunity of the skin (2, 14). Since the time when routine vaccination against smallpox ceased, the number of people with defects in cell-mediated immunity (transplant recipients, cancer chemotherapy patients, AIDS patients, etc.) has greatly increased. Persons who are known to be immunocompromised should be excluded from vaccination. However, individuals with undiagnosed immunosuppression may be vaccinated inadvertently. A case report of disseminated vaccinia in a human immunodeficiency virus-infected military recruit documents the consequences of such inadvertent vaccination (29). Also, individuals with eczema should be excluded from vaccination. People with eczema may, however, contract the virus (and the disease) from their close contacts. The number of patients with atopic dermatitis may, according to a "conservative estimate," have doubled or tripled since the era of smallpox vaccination (14).

It is obviously important to have an effective therapy at hand to treat complications of vaccination (12, 13, 27). We demonstrated previously that cidofovir is highly effective for both the treatment and prophylaxis of lethal vaccinia virus infections in intravenously infected SCID mice (25), and we also demonstrated the activity of 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine (S2242) and 5-iodo-2′-deoxyuridine (IDU) in this model (26, 28). Others confirmed and extended these findings (with cidofovir and S2242) using mice infected with vaccinia virus, cowpox virus, or a recombinant vaccinia-rabies glycoprotein virus vaccine (4, 5, 21, 30, 31). Interestingly, cidofovir (given systemically or via aerosol) also proved effective for mice that had been inoculated via the intranasal route or by aerosol (4, 5, 31). These models, however, do not mimic the complications of excessive replication of vaccinia virus, following vaccination against smallpox, in the skin of certain patients, such as those with an underlying immunodeficiency and those with ectopic dermatitis. Indeed, in these patients the virus spreads from the initial inoculation site in the skin to other parts of the body.

To establish a model for disseminated progressive vaccinia, we inoculated athymic nude (*nu*/*nu*) mice (which suffer from a severe deficiency in cell-mediated immunity) intracutaneously with vaccinia virus. Within a few days, typical vaccinia lesions developed at the site of virus inoculation. The virus replicated in the skin at the site of inoculation, as was evident from the detection of increasing levels of viral DNA in that tissue. By 2 weeks p.i., the infection disseminated to other parts of the skin. Intracutaneous inoculation also resulted in viremia and spread of the virus to the visceral organs as well as to the brain

TABLE 2. Effects of delayed start of topical treatment with cidofovir on cutaneous vaccinia virus infections in athymic nude mice

Treatment condition[a]	Effect[b]			
	MDLA	No. of mice with lesions/total no. of mice	MDD	No. of survivors/total no. of mice
Mock treatment	6.1 ± 0.9	9/9	34 ± 6.9	0/9
CDV (day 0–4 p.i.)	>127	0/9*****	>127	9/9*****
CDV (day 1–5 p.i.)	>127	0/4***	>127	4/4***
CDV (day 2–6 p.i.)	46.0	1/4*	>94[c]	3/4**
CDV (day 3–7 p.i.)	27 ± 7.0****	4/4	65 ± 43[d]	0/4
CDV (day 4–8 p.i.)	36 ± 17*	4/4	68 ± 13**	0/4

[a] Cidofovir (CDV; 50μl, 1% in DMSO) or DMSO alone (for the mock-treated group) was applied to the infected lumbosacral area once daily at the indicated days p.i.

[b] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$; *****, $P < 0.0001$. P values indicate significance of differences from the mock-treated group. The number of mice with lesions and the number of survivors were assessed on day 127 p.i., at which time the experiment was terminated.

[c] One animal died at day 94; the other three were terminated on day 127.

[d] One animal died early (at day 21 p.i.) because of trauma unrelated to the infection.

(although no obvious symptoms of encephalitis were noted). Since no detectable viremia was detected by use of a sensitive Q-PCR assay during the first 5 days p.i., it is as yet unclear how the virus disseminates. Autoinoculation is possible (and is likely the case for lesions on the head and feet, for example) but is likely not an explanation for lesions such as those on the back 2 cm or more away from the initial inoculation site. Also, detection of virus in visceral organs (and the brain) can be explained only by systemic spread of the virus. Immunocompetent hairless mice that had been inoculated in a similar way developed transient lesions; hence these mice could not be used to establish a model for disseminated progressive vaccinia. Smee and colleagues reported that hairless mice that had been immunosuppressed by cyclophosphamide and infected with the WR strain of vaccinia virus also developed a lethal disseminated vaccinia (D. F. Smee, K. W. Bailey, and R. W. Sidwell, Antivir. Res. **57:**A79, abstr. 129, 2003). Particular differences between the model of Smee and colleagues and the model reported here (such as differences in the aggressivity of the infection) may be explained by (i) the fact that different viral strains and inocula were used and (ii) differences in immunodeficiency status between cyclophosphamide-treated and athymic hairless mice, respectively.

Topical treatment with cidofovir proved highly effective, particularly when therapy was initiated within the first 2 days after infection. When treatment was initiated at a later time, a partial but still considerable protective effect was noted. This finding is consistent with the fact that the virus, following initial replication at the inoculation site (during which time topical treatment provides complete protection), is able to spread to the visceral organs, a location that cannot be reached (at least not at sufficiently high levels) by topically applied cidofovir. Systemic absorption of cidofovir following topical application is low (1). The fact that topical treatment with cidofovir, even when started as late as day 5 p.i., still resulted in an important protective effect indicates that the skin, at least during the first 5 days after the infection, remains (as is the case for smallpox vaccinees with complications) a major "platform" for the production and spread of new virus.

Of particular importance was our finding that systemically administered cidofovir is able to cause complete, or nearly complete, healing of disseminated vaccinia lesions in the

TABLE 3. Effects of systemically administered cidofovir on disseminated vaccinia lesions in athymic nude mice

Treatment condition[a]	Effect[b]			
	MDLH	No. of mice healed/total no. of mice	No. of mice improved/total no. of mice	MDD
Expt 1				
Mock treatment		0/3	0/3	35 ± 0.6
CDV (100 mg/kg, 6 times/wk)	13 ± 0.6	3/3	3/3	51 ± 6.2*
Expt 2				
Mock treatment		0/8	0/8	43 ± 8.8
CDV (100 mg/kg)				
5 times/wk	8.2 ± 3.3	4/5**	5/5****	87 ± 13****
3 times/wk	15 ± 11	4/4***	4/4***	90 ± 16**
2 times/wk	8	1/4	4/4***	60 ± 10*
1 time/wk		0/4	3/4*	62 ± 6.4***

[a] For experiment 1, treatment was initiated on day 15 p.i. (at which time mice had disseminated vaccinia) and was continued for 21 days during the next period of 25 days. For experiment 2, treatment was initiated on day 14 p.i. (at which time mice had disseminated vaccinia) and was continued either 1, 2, 3, or 5 times a week either for the next 6 weeks (for the 5-times-per week group; treatment stopped at day 51 p.i.) or for 7 consecutive weeks (for all other groups; treatment stopped at day 63 p.i.).

[b] Counting for MDLH begins at the start of therapy; counting for MDD begins at infection. Healed, complete disappearance of lesions (only some scarring may remain). Improved, obvious improvement in the number and severity of the lesions (includes those animals whose lesions have completely healed). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$. P values indicate significance of differences from the mock-treated group.

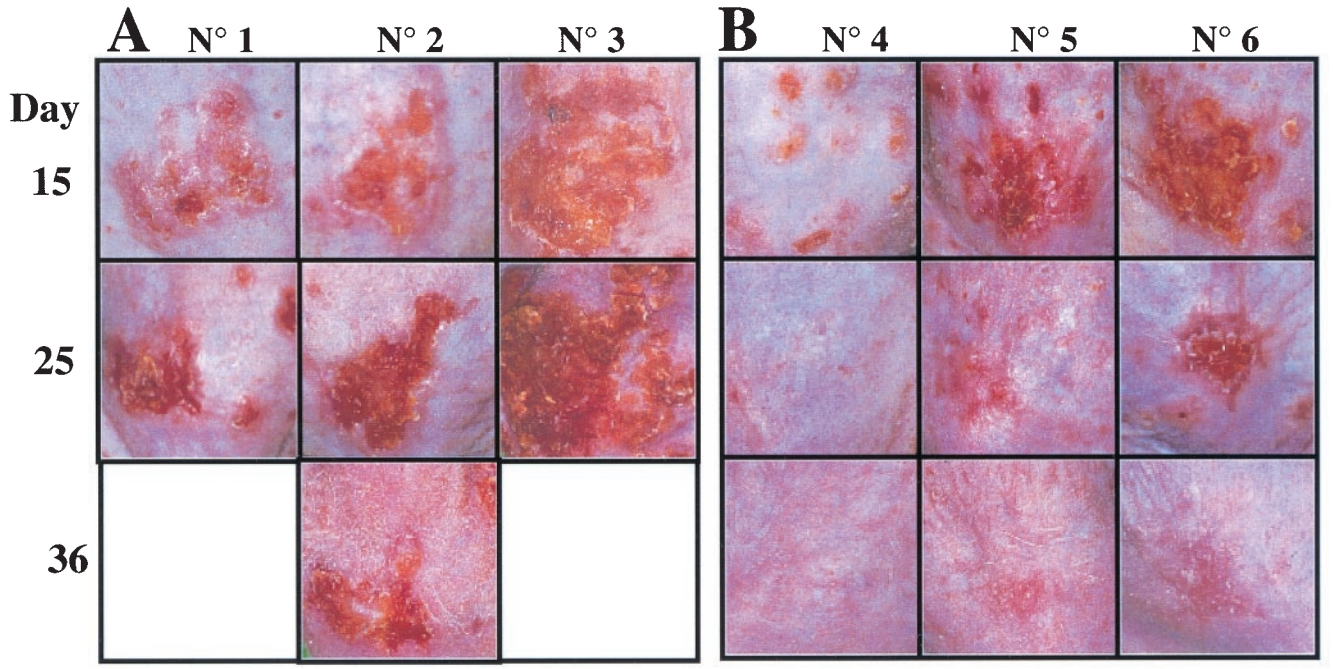

FIG. 3. Effect of systemically administered cidofovir on disseminated vaccinia when therapy was initiated on day 15 p.i. (A) Progression of cutaneous vaccinia in three mock-treated animals. Mice 1 and 3 died on day 35 p.i.; mouse 2 died on day 36 p.i. (B) Effect of subcutaneous treatment with cidofovir (at 100 mg/kg/day), initiated on day 15 p.i., on disseminated cutaneous vaccinia lesions. Cidofovir was given for 21 days (over a period of 24 days).

model presented here. Even infrequent dosing (1, 2, or 3 times/week) resulted in an improvement or healing of the lesions and markedly delayed virus-induced mortality. Following cessation of therapy, however, the virus recurred, not a surprising observation with a severely immunocompromised host. In an earlier study of vaccinia virus-infected SCID mice that had been treated with cidofovir over about 140 days, no resistant virus was detected (25; our unpublished data). It is therefore unlikely that the vaccinia virus in the present study would have become resistant to cidofovir.

It must be noted that the doses of systemically administered cidofovir that are required for efficacy against cutaneous vaccinia virus infection are markedly higher than those needed to curtail vaccinia pox tail lesions in NMRI mice or systemic vaccinia virus or cowpox virus infections in SCID mice (4, 25, 30). Thus, higher doses of cidofovir may be needed to produce active antiviral concentrations of the compound in the skin than in other organs. Commonly the highest dose of cidofovir that is given to humans is 5 mg/kg/week. Clearance of cidofovir is 0.6 liter/h/kg in rats (9), 0.21 liter/h/kg in monkeys (10), and 0.15 liter/h/kg in humans (11). There are no published values for mice. By allometric scaling, a clearance of 1.1 liters/h/kg ($r = 0.9994$) can be estimated for mice. By using the estimated clearance in the mouse, the relative equivalent doses for these species, in terms of creating equivalent areas under the concentration-time curve (AUC), are 7.69 mg/kg for mice, 4 mg/kg for rats, 1.4 mg/kg for monkey, and 1 mg/kg for humans. In other words, giving a 1-mg/kg dose to a human, a 1.4-mg/kg dose to a monkey, a 4-mg/kg dose to a rat, and a 7.69-mg/kg dose to a mouse would all result in the same exposure in terms of AUC. Therefore, one dose a week of, for example, 100 mg of cidofovir/kg for a mouse (a dose that delayed virus-induced mortality by 27 days [Table 2]) would be equivalent in terms of AUC to a 6.5-mg/kg dose in humans, which is close to the currently used dose of 5 mg/kg.

The murine model described here mimics progressive disseminated cutaneous vaccinia in humans and, at the same time, allows demonstration of the efficacy of cidofovir in curtailing the disease. The clinical efficacy of cidofovir has been demonstrated, following either topical application or intravenous administration of the drug, in patients with severe infections caused by other members of the family *Poxviridae*, i.e., molluscum contagiosum and ecthyma (sheep pox, orf) (20, 22, 24, 32, 34; E. G. Davies, A. Thrasher, K. Lacey, and J. Harper, Letter, Lancet **353:**2042, 1999). Our present findings, together with the evidence that cidofovir is effective against molluscum contagiosum (even following intravenous dosing) and orf in the clinical setting, provide evidence that cidofovir may be effective in the treatment of complications of vaccination against smallpox resulting from excessive replication of the vaccinia virus.

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